NEW PHOSPHORAMIDITE COMPOUNDS

Field of the Invention

The present invention relates to a phosphoramidite compound which can be advantageously used to synthesize DNA variants.

Background of the Invention

There have been attempts to develop a drug based on a DNA synthesized by a chemical method (Agrawal, S., *Synthesis and Properties*, Humana Press: Totowa, Chapter 1-4, (1993) and Kool, E. T., *Chem. Rev.*, 97; 1473(1997)). Also, various efforts have been made to develop modified DNAs having interesting structural features (Newcome, G. R., et al., *Dendritic Molecules: Concepts, Synthesis, Perspectives*, VCH Publishers, New York, 116 (1996); Shchepinov, M. S. et al., *Nucleic Acids Res.*, 25, 4447-4454 (1997); Shchepinov, M. S. et al., *Nucleic Acids Res.*, 27, 3035-3041(1999); and Winfree, E. et al., *Nature*, 394, 539-544(1998)).

In this line, branched DNAs (bDNAs) composed of several oligodeoxyribonucleotide (ODN) strands have been synthesized and studied in order to elucidate the structural and biological characteristics of wild-type bDNAs of interest (Hudson, R. H.; Uddin, A. H.; Damha, M. J., *J. Am. Chem. Soc.*, 117, 12470-12477(1995); and Collins, M. L. et al., *Nucleic Acids Research*, Vol. 25, No. 15, 2979-2984(1997); and Horn, T, et al., *Nucleic Acids Research*, Vol. 25, No. 23, 4835-4849(1997)). Such branched DNAs are sometimes used to synthesis a hyperbranched polymer or dendrimer. The dendrimer may be modified by introducing various functional groups at the branch ends thereof so that it can attain a specific biological function (Newkome, G. R. et al., *Chem. Rev.*, 99: 1689-1746(1999)).

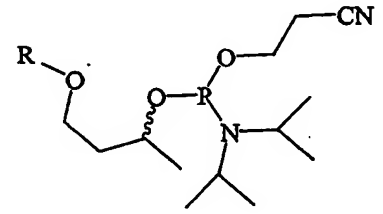
Phosphoramidite compounds coupled with adenosine (A), guanosine (G), cytidin (C) or thymidin (T), have been used to synthesize various wild type DNAs using a DNA synthesizer and such phosphoramidite derivatives can be easily inserted into DNA.

Accordingly, the present inventors have endeavored to develop a new functional phosphoramidite compound, which can be usefully used in synthesizing various oligodeocyribonucleotides (ODNs).

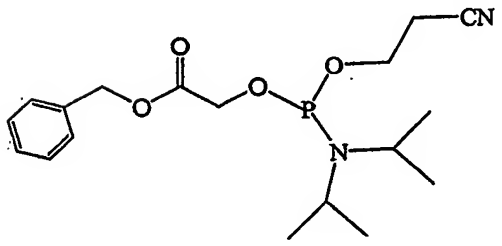
Summary of the Invention

Accordingly, it is an object of the present invention to provide a novel phosphoramidite compound which can be used in the synthesis of a desired oligodeocyribonucleotide.

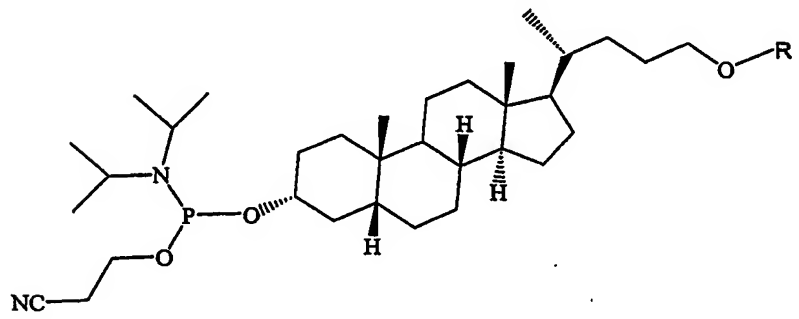
In accordance with an aspect of the present invention, there is provided a phosphoramidite compound of formula (I), (II), (III), (IV) or (V):



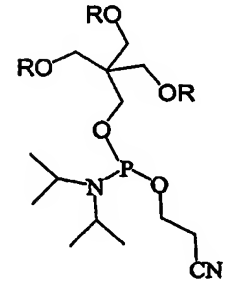
(I)



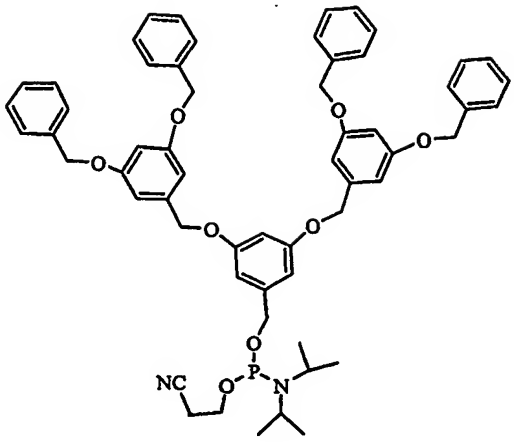
(II)



(III)



(IV)



(V)

wherein:

R is a dimethoxytrityl (DMTr), levulinyl (Lev) or tert-butyldimethylsilyl (TBDMS) group.

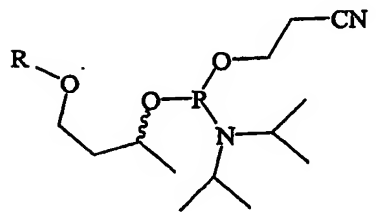
Brief Description of the Drawings

The above and other objects and features of the present invention will become apparent from the following description of the invention taken in conjunction with the following accompanying drawings, wherein:

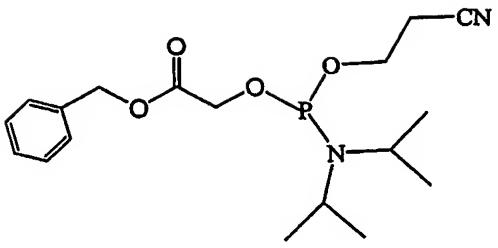
Fig. 1 shows the melting temperatures of synthesized oligomers;
Figs. 2 and 3 illustrate the CD spectra of synthesized oligomers; and
Fig. 4 are HPLC scans of purified oligomers.

Detailed Description of the Invention

The phosphoramidite compound of the present invention is a compound represented by one of formula (I) to (V):



(I)



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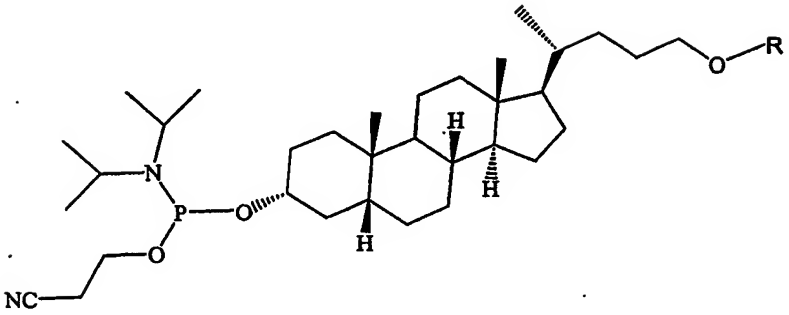
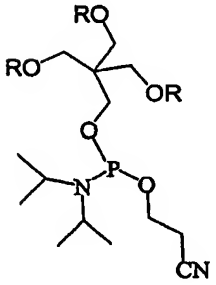
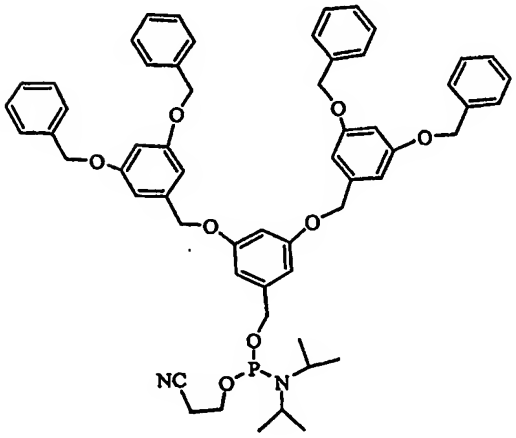


(III)



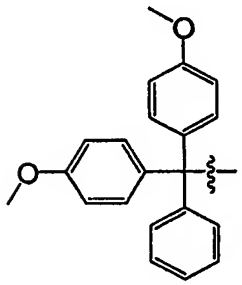
(IV)



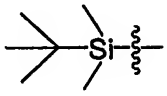
(V)

wherein:

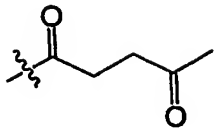
R is a dimethoxytrityl (DMTr), levulinyl (Lev) or tert-butyldimethylsilyl (TBDMS) group.



DMTr

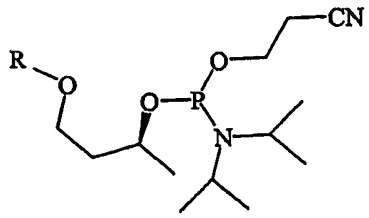


TBDMS

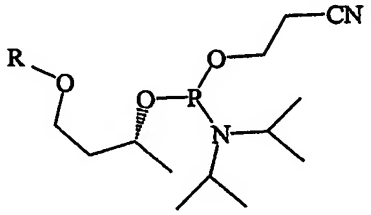


Lev

The inventive phosphoramidite compound of formula (I) includes its (S)- and (R)-isomers:



(S)-isomer



(R)-isomer

Among the phosphoramidite compounds of the present invention, preferred are:

(*S*)-(+)-1-O-DMTr-3-O-(2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite)-1,3-butanediol;

(*R*)-(-)-1-O-DMTr-3-O-(2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite)-1,3-butanediol;

O-((2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite)-benzylglycolate;

O-DMTr-((2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite)-lithocholic alcohol;

O-tri-DMTr-((2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite)-pentaerithritol;

O-DMTr-O-di-Lev-O-((2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite)-pentaerithritol;

O-DMTr-O-Lev-O-TBDMS-((2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite)-pentaerithritol; and

dendrimer phosphoramidite compound of formula (V).

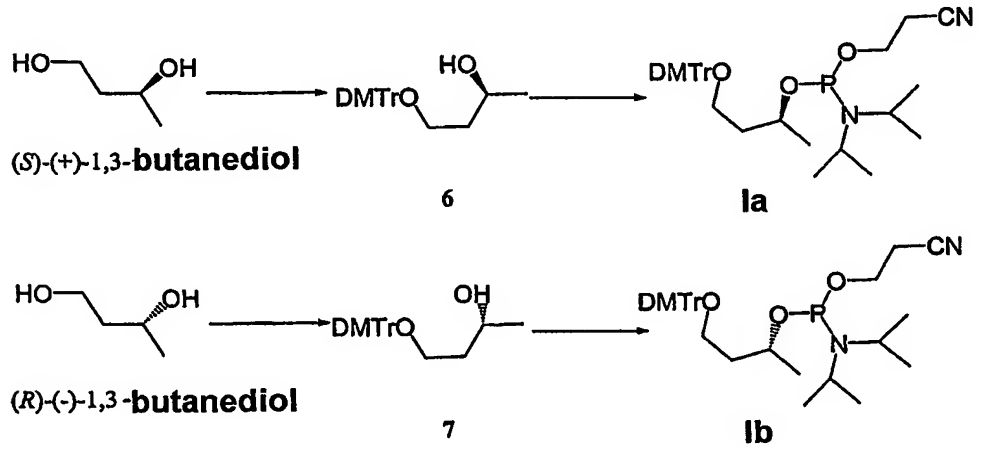
The inventive phosphoramidite compound may be prepared by introducing a desired functional group, e.g., 1.3-butanediol, benzyl glycolate or lithocolic acid as described below. A phosphoramidite compound prepared by introducing pentaerithritol or dendrimer, in particular, can be advantageously used in the synthesis of functional branched DNAs (bDNAs).

1) Preparation of an optically pure phosphoramidite compound of formula (I) using (*S*)-(+)- or (*R*)-(-)-1,3-butanediol (Scheme 1)

(*S*)-(+)- or (*R*)-(-)-phosphoramidite can be prepared by protecting the 1-hydroxyl group of (*S*)-(+)- or (*R*)-(-)-1,3-butanediol with a DMtr group to obtain compound 6 or 7 and introducing the phosphoramidite group into the secondary hydroxyl group to obtain the (S)-(+)- or (R)-(-)-phosphoramidite of formula (Ia) or (Ib).

The inventive (S)-(+)- or (R)-(-)-phosphoramidite may be used in linking oligonucleotides as a linker.

Scheme 1

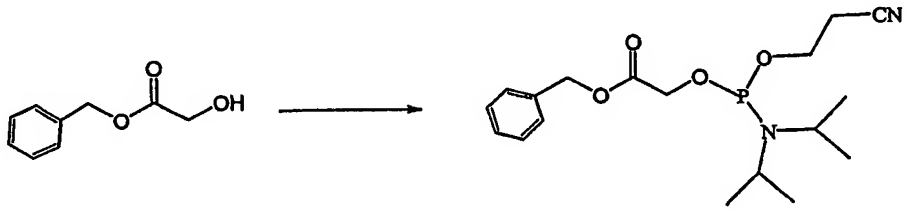


2) Preparation of the phosphoramidite compound of formula (II) using benzyl glycolate (Scheme 2)

The phosphoramidite compound of formula (II) can be prepared by reacting benzyl glycolate and chloro-(2-cyanoethyl)-*N,N*-diisopropyl-phosphine in THF in the presence of DIPEA (*N,N*-diisopropylethylamine).

The inventive phosphoramidite compound of formula (II) may be used for introducing an acidic functional group into an oligonucleotide.

Scheme 2



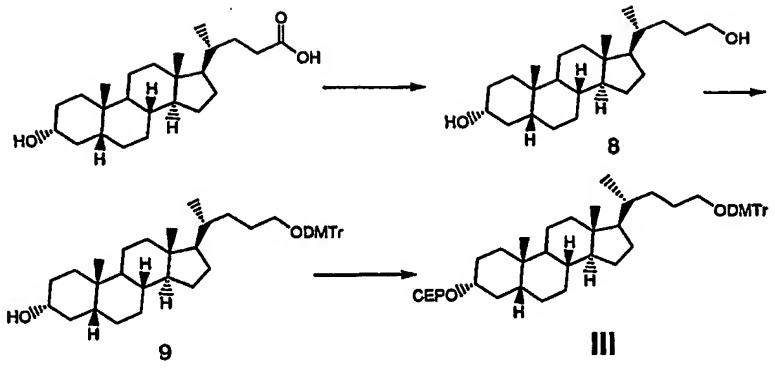
II

3) Preparation of the phosphoramidite compound of formula (III) using lithocholic acid (Scheme 3)

The phosphoramidite compound of formula (III) can be prepared by reducing the carboxyl group of lithocholic acid to obtain compound 8, protecting the primary hydroxyl group with a DMTr group, and introducing the phosphoramidite group into the secondary hydroxyl group.

The inventive phosphoramidite compound of formula (III) has an enhanced cell permeability due to the hydrophobic lithocholic acid residue and therefore, it may be used in gene therapy. Further, the inventive phosphoramidite compound of formula (III) is expected to be useful in modifying the secondary and third structure of a DNA.

Scheme 3

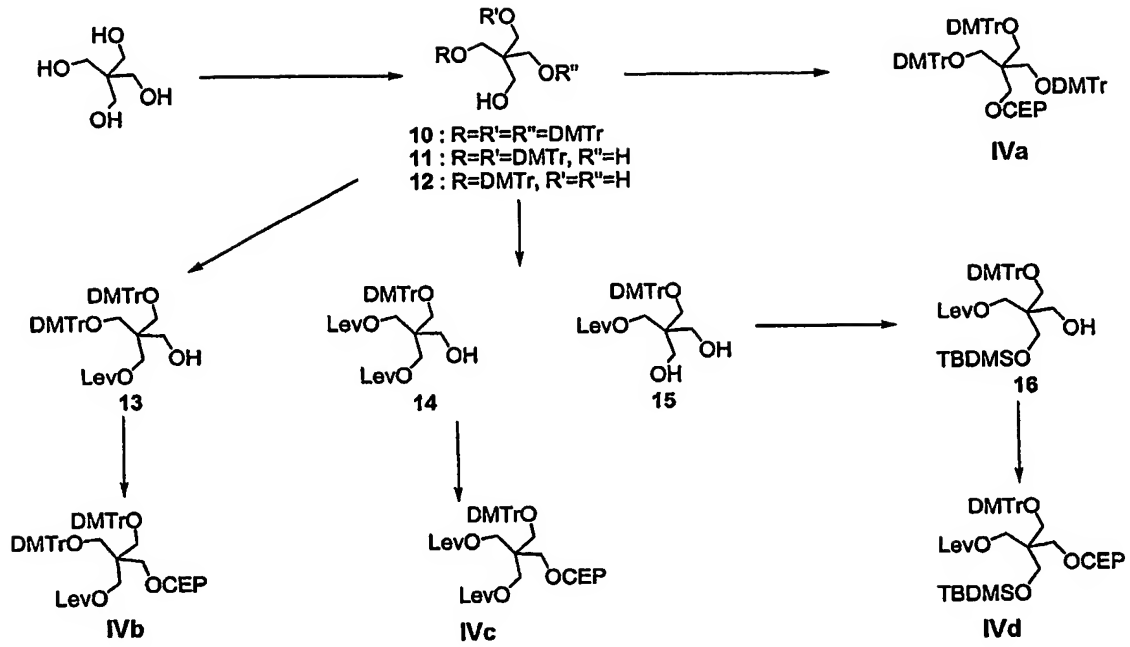


4) Preparation of the phosphoramidite compound of formula (IV) using pentaerythritol (Scheme 4)

The phosphoramidite compound of formula (IVa) can be prepared by protecting three of the four hydroxyl groups of pentaerythritol with DMTr groups to obtain compound 10 and introducing the phosphoramidite group into the remaining hydroxyl group. The phosphoramidite compound of formula (IVb) can be prepared by protecting two of the four hydroxyl groups of pentaerythritol with DMTr groups to obtain compound 11, protecting a third hydroxyl group with an Lev group and introducing the phosphoramidite into the remaining hydroxyl group. The phosphoramidite compound of formula (IVc) can be prepared by protecting the hydroxyl group of pentaerythritol with a DMTr group to obtain compound 12, protecting two other hydroxyl groups with Lev groups to obtain compound 14 and introducing the phosphoramidite into the remaining hydroxyl group. Further, the phosphoramidite compound of formula (IVd) can be prepared by introducing a TBDMS (tert-butyldimethylsilyl) group into the compound 15 to obtain compound 12 and introducing the phosphoramidite into the remaining hydroxyl group.

The inventive phosphoramidite compound of formula (IV) may be used in synthesizing dendrimers and bDNAs, especially in synthesizing bDNAs having different base sequences or nano structural oligodeoxyribonucleotides.

Scheme 4

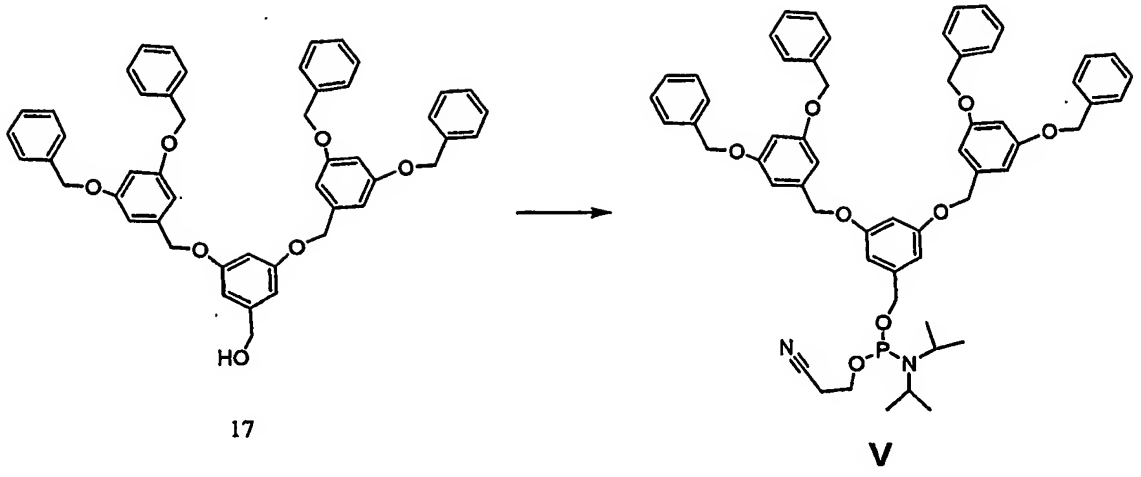


5) Preparation of the dendrimer phosphoramidite compound of formula (V) using dendrimer (Scheme 5)

The dendrimer phosphoramidite compound of formula (V) can be prepared by introducing the phosphoramidite group into the hydroxyl group of dendrimer compound 17

The inventive dendrimer phosphoramidite compound of formula (V) may be used in introducing a dendrimer having desired functional groups into an oligonucleotide.

Scheme 5



17 V

The following examples are intended to further illustrate the present invention without limiting its scope.

Example 1: Preparation of the phosphoramidite compound of formula (Ia) using (*S*)-(+)-1,3-butanediol

(1) Preparation of *S*-(+)-1-*O*-(4,4'-dimethoxytrityl)-1,3-butanediol (compound 6)

S-(+)-1,3-butanediol (96 mg, 1.065 mmol) in 3 ml of pyridine was cooled in an ice-water bath and 4,4'-dimethoxytrityl chloride (430 mg, 1.27 mmol) was added thereto. The resulting mixture was stirred for 6 hours at room temperature. 10 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 15 ml of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting yellow liquid residue was purified by silica gel column chlomatography (eluent - ethyl acetate:hexane = 1 : 3) to obtain the title compound (401 mg, 1.02 mmol) in a yield of 96%.

R_f= 0.3 (ethyl acetate : Hexane = 1 : 2); IR (NaCl) ν (cm^{-1}) 3462, 3059, 3034, 2959, 2927, 2848, 2835, 1607, 1508, 1250; ^1H NMR (Acetone-d_6) δ 7.49 (br, 1H), 7.46(br, 1H), 7.36-7.18 (m, 7H), 6.86 (t, 2H, *J*=2.6Hz), 6.84 (t, 2H, J=2.6Hz), 3.93(br, 1H), 3.73(s, 6H), 3.50(br, 1H), 3.28-3.14 (m,

2H), 1.73 (m, 2H), 1.11(d, 3H, *J*=6.2Hz); ^{13}C-NMR (75.5 MHz, Acetone-d_6) δ 158.1, 145.3, 136.1, 136.0, 129.5, 127.6, 127.2, 126.1, 112.5, 85.4, 64.2, 60.6, 54.2, 39.0, 23.1; MS-FAB (m/z): $[M]^+$ calcd for $C_{25}H_{28}O_4$, 392; found 392.; $[\alpha]^{21}_D = +17.6$ (c 1.0, $CHCl_3$).

(2) Preparation of *S*-(+)-1-*O*-DMTr-3-O-((2-cyanoethyl)-N,N-dimethoxytrityl)-1,3-butanediol (Compound Ia)

S-(+)-1-*O*-(4,4'-dimethoxytrityl)-1,3-butanediol (158 mg, 0.402 mmol) obtained in step 1 was dissolved in 3 ml of THF, DIPEA (140 μℓ, 0.804 mmol) was added thereto, and then the mixture was stirred for 30 min, followed by adding chloro-(2-cyanoethyl)-*N*,*N*-diisopropylamino phosphine (177 μℓ, 0.80 mmol) dropwise thereto. Then, the white precipitates formed were filtered and dried under a reduced pressure. 20 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting yellow liquid residue was purified by silica gel column chlomatography (eluent - ethyl acetate : hexane = 1 : 5) to obtain the title compound (203 mg, 0.34 mmol) as a colorless oil in a yield of 85%.

^{1}H-NMR (300 MHz, Acetone-d_6) δ 7.47-7.43(2H, m), 7.34-7.25 (5H, m), 7.22-7.16 (1H, m), 6.89-6.80 (4H, m), 4.15 (1H, m), 3.74 (3H, s), 3.73 (3H, s), 3.63-3.51 (3H, m), 3.20-3.16 (2H, m), 2.68 (1H, t, *J*=6.0Hz), 2.55 (1H, t, *J*=6.0Hz), 1.94-1.73 (3H, m), 1.21-1.11 (12H, m), 1.07 (1.5H, s), 1.05 (1.5H, s); ^{13}C-NMR (75.5 MHz, Acetone-d_6) δ 158.1, 145.2, 136.0, 129.6, 129.5, 127.7, 127.6, 127.2, 126.1, 117.7, 117.6, 112.5, 85.4, 68.0, 67.7, 67.4, 67.2, 60.0, 59.8, 59.2, 58.1, 57.8, 57.5, 54.2, 42.4, 42.2, 38.3, 23.7, 23.6, 23.6, 23.5, 23.4, 21.6, 19.5, 19.4; ^{31}P-NMR (121 MHz, Acetone-d_6) δ 149.0, 148.3; MS-FAB (m/z): $[M+Na]^+$ calcd for $C_{34}H_{45}N_2O_5P_1Na_1$, 615; found 615.

Example 2: Preparation of the phosphoramidite compound of formula (Ib) using (*R*)-(-)-1,3-butanediol

(1) Preparation of *R*-(-)-1-*O*-(4,4'-dimethoxytrityl)-1,3-butanediol (compound 7)

R-(-)-1,3-butanediol (103 mg, 1.14 mmol) in 3 ml of pyridine was

cooled in an ice-water bath, and 4,4'-dimethoxytrityl chloride (460 mg, 1.36 mmol) was added thereto. The resulting mixture was stirred for 6 hours at room temperature. 10 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 15 ml of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting yellow liquid residue was purified by silica gel column chlomatography (eluent - ethyl acetate:hexane = 1 : 3) to obtain the title compound (437 mg, 1.11 mmol) in a yield of 97%.

R_f= 0.3 (ethyl acetate : hexane = 1 : 2); IR (NaCl) v (cm^{-1}) 3462, 3059, 3034, 2960, 2929, 2835, 1607, 1508, 1250; ^1H NMR (Acetone-d_6) δ 7.47 (t, 1H, *J*=1.7Hz), 7.45(br, 1H), 7.35-7.20 (m, 7H), 6.87 (t, 2H, *J*=2.6Hz), 6.84 (t, 2H, J=2.6Hz), 3.92(br, 1H), 3.73(s, 6H), 3.47(d, 1H, *J*=3.7Hz), 3.25-3.14 (m, 2H), 1.71 (m, 2H), 1.09(d, 3H, *J*=6.2Hz); ^{13}C-NMR (75.5 MHz, Acetone-d_6) δ 158.1, 145.2, 136.1, 136.0, 129.5, 127.6, 127.2, 126.1, 112.5, 85.4, 64.2, 60.5, 54.1, 38.9, 23.0; MS-FAB (m/z): $[M]^+$ calcd for $C_{25}H_{28}O_4$, 392; found 392.; $[\alpha]^{21}_D$ = -9.9 (c 1.0, $CHCl_3$).

(2) Preparation of *R*-(-)-1-*O*-DMTr-3-O-((2-cyanoethyl)-*N,N*-dimethoxytrityl)-1,3-butanediol

R-(-)-1-*O*-(4,4'-dimethoxytrityl)-1,3-butanediol (138 mg, 0.315 mmol) obtained in step 1 was dissolved in 2 ml of THF, DIPEA (140 ㎕, 0.804 mmol) was added thereto and stirred for 30 min, followed by adding chloro-(2-cyanoethyl)-*N,N*-diisopropylamino phosphine (157 ㎕, 0.70 mmol) dropwise thereto. Then, white precipitates formed were filtered, dried under a reduced pressure. 10 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 15 ml of CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting yellow liquid residue was purified by silica gel column chlomatography (eluent - ethyl acetate : hexane = 1 : 5) to obtain the title compound (108 mg, 0.182 mmol) as a colorless oil in a yield of 52%.

R_f= 0.45 (ethyl acetate:hexane=1:5) ^1H-NMR (300 MHz, Acetone-d_6) δ 7.47-7.43(2H, m), 7.34-7.25 (5H, m), 7.22-7.16 (1H, m), 6.89-6.80 (4H, m), 4.15 (1H, m), 3.76 (3H, s), 3.75 (3H, s), 3.63-3.51 (3H, m), 3.20-3.16 (2H, m), 2.68 (1H, t, *J*=6.0Hz), 2.55 (1H, t, *J*=6.0Hz), 1.94-1.73 (3H, m),

1.19-1.10 (12H, m), 1.05 (1.5H, s), 1.03 (1.5H, s); ^{13}C-NMR (75.5 MHz, Acetone-d_6) δ 158.1, 145.2, 136.0, 129.5, 129.5, 127.6, 127.5, 127.2, 126.1, 117.6, 112.4, 85.3, 67.9, 67.7, 67.4, 67.2, 66.7, 59.9, 59.8, 58.0, 57.8, 57.5, 54.1, 42.4, 42.2, 38.3, 38.2, 24.8, 23.7, 23.6, 23.5, 23.4, 23.3, 21.6, 19.4, 19.3; ^{31}P-NMR (121 MHz, Acetone-d_6) δ 149.0, 148.3; MS-FAB (m/z): $[M+Na]^+$ calcd for $C_{34}H_{45}N_2O_5P_1Na_1$, 615; found 615.

Test Example 1

The effect exerted by the chiral structural difference of enantiomers on the oligonucleotide structure was examined as follows.

The compounds of formula (Ia) and (Ib) obtained Example 1 and 2 respectively, were each inserted into an oligonucleotide using Expedite Nucleic Acid Synthesis System 8090 and the molecular weight of synthesized ODNs was determined by employing Maldi-Tof mass(Acceleratting voltage: 25000V, Matrix: 3-Hydroxypicolinic acid, Polarity: Positive).

The results are shown in Table 1.

Table 1

No.	Sequence	The molecular weight	
		calcd	Found
1	5'd-T_6**Rp**T_5	3425.3	3433.3
2	5'd-T_6**Sp**T_5	3425.3	3462.3
3	5'd-A_5**Rp**A_6	3528.7	3534.5
4	5'd-A_5**Sp**A_6	3528.7	3538.6
5	5'd-T_{12}	3577.4	3594.0
6	5'd-T_{11}	3274.2	3277.6
7	5'd-A_{12}	3690.2	3701.0
8	5'd-A_{11}	3377.6	3384.1
9	5'd-AACGTT**Rp**AACGTT	3786.0	3794.0
10	5'd-AACGTT**Sp**AACGTT	3786.1	3794.9
11	5'd-AACGTTAAACGTT	3947.6	3950.7
12	5'd-AACGTTTAACGTT	3938.2	3944.7
13	5'd-AACGTTGAACGTT	3963.2	3969.1
14	5'd-AACGTTCAACGTT	3923.2	3925.9

Rp: *R*-(+)-1-*O*-DMTr-3-O-((2-cyanoethyl)-*N,N*-dimethoxytrityl)-1,3-butanediol
Sp: *S*-(+)-1-*O*-DMTr-3-O-((2-cyanoethyl)-*N,N*-dimethoxytrityl)-1,3-butanediol

Further, the melting temperatures (Tm) of various duplexes (oligo / oligo) were determined by measuring the changes in the absorbance at 260 nm (cuvette, 1 Cm path length) with increasing temperature at rate of 1.0 ℃/min using solution in Tris-HCl buffer (10 mM, pH 7.2) containing 100 mM NaCl and 20 mM $MgCl_2$. The result is shown in Table 1 and Fig. 1. The total concentrations of duplexes 1, 2 and 9 were each adjusted to 4.0 μ M, while the total concentrations of duplexes 3 to 8 and 10, to 6.6 μ M.

Table 2

Entry	Duplex	Sequence	Tm (°C)	ΔTm (°C)
1	Oligo 1 / Oligo 7	5'd-T_6**Rp**T_5 / 5'd-A_{12}	26 °C	-12 °C
2	Oligo 2 / Oligo 7	5'd-T_6**Sp**T_5 / 5'd-A_{12}	26 °C	-12 °C
3	Oligo 3 / Oligo 5	5'd- A_5**Rp**A_6 / 5'd-T_{12}	28 °C	-10 °C
4	Oligo 4 / Oligo 5	5'd- A_5**Sp**A_6 / 5'd-T_{12}	27 °C	-11 °C
5	Oligo 1 / Oligo 3	5'd-T_6**Rp**T_5/ 5'd- A_5**Rp**A_6	15 °C	-23 °C
6	Oligo 1 / Oligo 4	5'd-T_6**Rp**T_5 / 5'd- A_5**Sp**A_6	14 °C	-24 °C
7	Oligo 2 / Oligo 3	5'd-T_6**Sp**T_5 / 5'd- A_5**Rp**A_6	15 °C	-23 °C
8	Oligo 2 / Oligo 4	5'd-T_6**Sp**T_5 / 5'd- A_5**Sp**A_6	15 °C	-23 °C
9	Oligo 5 / Oligo 7	5'd-T_{12} / 5'd-A_{12}	38 °C	0 °C
10	Oligo 6 / Oligo 8	5'd-T_{11} / 5'd-A_{11}	35 °C	-3 °C

As shown in Table 2, Tm (℃) values of the duplex (oligo 1/oligo 7) and duplex (oligo 2/oligo 7) are lower by about 12 °C than that of duplex (oligo 5/oligo 7). Such negative ΔTm value is caused by the substitution of the nucleoside with 1,3-butanediol which has higher flexibility than a sugar moiety and no base that can hydrogen bond to the oligonucleotide. This result suggests that the difference in the chiral structure of a pair of enantiomers does not affect Tm value of the oligonucleotide.

Further, CD (circular dichroism) spectra of the oligomers are shown in Figs. 2 and 3. As can seen from these results, the double helix structure of the inventive oligomer is similar to that of a wild-type oligomer.

HPLC (high performance liquid chromatography) was also carried out for oligo 1, oligo 2 and a mixture thereof (1:1) under the following conditions.

- temperature: room temperature
- column: Agilent Eclipse XDB-C18, 4.6 x 150 mm, 5 μ , 80 Å pore size
- solvent program : 5% acetonitrile/0.1M triethylammonium acetate (TEAA) (pH 7.0) was eluted for 10 min. Then, the gradient was linearly

increased to 50% acetonitrile/0.1M TEAA for 10 min. After 5 minutes, the gradient was linearly recovered to the initial state.

The result in Fig. 4 shows that oligo 1(a), oligo 2(b) and a mixture thereof(c) eluted at the same retention time, suggesting that the structural difference in terms of (S)- and (R)-isomers does not significantly affect the oligonucleotide structure.

Example 3: Preparation of O-((2-cyanoethyl)-*N,N*-diisopropyl-phosphoramidite)-benzylglycolate using benzylglycolate

Benzyl glycolate (100 $\mu\ell$, 0.704 mmol) and DIPEA (480 $\mu\ell$, 2.8 mmol) were added to 7 ml of THF. After stirring for 30 min, chloro-(2-cyanoethyl)-*N,N*-diisopropyl-phosphine (234 $\mu\ell$, 1.06 mmol) was added dropwise thereto and stirred for 30 min. Then, the bulky white precipitates formed were filtered and dried under a reduced pressure. 25 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 40 ml of CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting yellow liquid residue was purified by silica gel column chlomatography (eluent - ethyl acetate : hexane = 1 : 3) to obtain the title compound (167 mg, 0.458 mmol) as a colorless liquid in a yield of 65%.

MS (FAB): m/z: 389.0 [M+Na^+]; IR (neat): v=3032, 2967, 2932, 1758, 1496, 1455, 1395, 1185, 1098; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.33 (5H, s), 5.16 (2H, s), 4.28-4.17 (2H, m), 3.91-3.81 (2H, m), 3.64-3.57 (2H, m), 2.63-2.56 (2H, m), 1.77-1.23 (12H, m); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 169.8, 169.8, 134.9, 128.1, 128.0, 117.3, 66.3, 60.4, 60.1, 58.6, 58.4, 42.9, 42.7, 24.2, 24.1, 24.0, 19.8, 19.8; ^{31}P-NMR (121 MHz, $CDCl_3$) δ 153.7; HRMS-FAB (m/z): [M+Na]$^+$ calcd for $C_{18}H_{27}N_2O_4P_1Na_1$, 389.1606; found, 368.1603.

Example 4: Preparation of phosphoramidite compound of formula (III) using lithocholic alcohol

(1) Preparation of lithocholic alcohol (compound 8)

Lithocholic acid (527 mg, 1.40 mmol) was dissolved in 30 ml of

THF and cooled to 0 ℃, followed by adding LAH (lithium aluminum hydride, 247.6mg, 6.58mmol) dropwise thereto. After stirring for 4 hours, 250 $\mu\ell$ of H_2O and 250 $\mu\ell$ of 15% NaOH were sequentially added thereto, followed by adding 750 $\mu\ell$ of H_2O thereto. Then, the bulky white precipitates formed were filtered and dried under a reduced pressure to obtain the title compound (486.4mg, 1.33 mmol) as a white solid in a yield of 95%.

m.p. 96.5-97.8°C; MS (EI): m/z: 362.3 [M^+]; IR (neat): v=3205, 2934, 2862, 1446, 1066, 914, 728cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ=3.61-3.57 (3H, m), 1.82-1.01 (28H, m), 0.90 (6H, s), 0.62 (3H, s); ^{13}C-NMR (75.5MHz, $CDCl_3$) δ 70.3, 62.0, 56.0, 55.7, 42.1, 41.6, 35.9, 35.3, 35.1, 35.0, 34.1, 31.5, 30.0, 29.0, 27.8, 26.8, 26.0, 23.7, 23.0, 20.3, 18.2, 11.6; HRMS-FAB (m/z): [M-OH]$^+$ calcd for $C_{24}H_{41}O_1$, 345.3157; found, 345.20.

(2) Preparation of *O*-DMTr-lithocholic alcohol (compound 9)

Lithocholic alcohol (455.1 mg, 1.25 mmol) obtained in step 1 and DMAP (68 mg, 0.06 mmol) were dissolved in 10 ml of pyridine, DMTr-Cl (544 mg, 1.63 mmol) was added thereto and stirred for 19 hours at room temperature. 50 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 50 ml of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting orange colored oil residue was purified by silica gel column chromatography (eluent - ethyl acetate : hexane = 1 : 4) to obtain the title compound (744.8 mg, 1.12 mmol) as a white solid in a yield of 89%.

m.p. 81.2-82.1°C; MS (FAB): m/z: 664.4 (M^+); IR (neat): v=3421, 2934, 2863, 1739, 1608, 1582, 1509, 1446, 1250, 1175, 1036, 827cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ=7.45 (d, 2H, J=7.2Hz), 7.35-7.26 (m, 7H), 6.89-6.80 (dd, 4H, J_1=7.0Hz, J_2=1.9Hz), 3.80 (s, 6H), 3.64 (br, 1H), 3.04-2.98 (m, 2H), 1.99-0.89 (m, 34H), 0.63 (s, 3H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ=159.0, 148.2, 137.6, 130.7, 129.0, 128.3, 127.2, 126.6, 113.7, 86.4, 72.6, 64.7, 57.3, 56.9, 55.9, 43.4, 42.9, 41.2, 40.9, 37.2, 36.6, 36.3, 36.1, 35.3, 33.1, 31.3, 28.9, 27.9, 27.4, 27.2, 24.9, 24.1, 21.6, 19.4, 12.8; HRMS-FAB (m/z): [M-OH]$^+$ calcd for $C_{45}H_{60}O_4$, 664.4492; found, 664.4489.

(3) Preparation of *O*-DMTr-((2-cyanoethyl)-*N,N*-diisopropyl-phosphoramidite)-lithocholic alcohol (compound III)

O-DMTr-lithocholic alcohol (89.2 mg, 0.15 mmol) obtained in step 2 and DIPEA (77 $\mu\ell$, 0.45 mmol) were dissolved in 2 ml of CH_2Cl_2. Chloro-(2-cyanoethyl)-*N,N*-diisopropyl-phosphine (49 $\mu\ell$, 0.225 mmol) was added dropwise thereto and stirred for 15 min at room temperature. 10 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 10 ml of CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting white solid residue was purified by flash chromatography (eluent - CH_2Cl_2 : hexane = 1 : 1.5) to obtain the title compound (69.3 mg, 0.081 mmol) as a white solid in a yield of 54%.

MS (FAB): m/z: 866 [M+H$^+$]; IR (neat): v=3353, 2962, 2935, 2866, 1608, 1509, 1463, 1446, 1376, 1364, 1300, 1250, 1178, 1035, 975, 827, 754cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$) δ=7.35 (d, 2H, *J*=7.6Hz), 7.25-7.11 (m, 7H), 6.73 (d, 4H, *J*=8.7Hz), 3.70 (s, 9H), 3.52 (m, 2H), 2.91 (m, 2H), 2.65 (t, 2H, *J*=6.4Hz), 1.88-0.80 (m, 46H), 0.53 (s, 3H); ^{13}C-NMR (75.5MHz, $CDCl_3$) δ=159.0, 146.2, 137.6, 130.7, 129.0, 128.3, 127.2, 113.7, 110.1, 86.4, 77.9, 75.2, 74.9, 64.7, 59.1, 58.8, 57.2, 56.9, 55.9, 43.8, 43.7, 43.4, 43.0, 41.1, 40.9, 36.6, 36.2, 36.0, 35.3, 33.1, 32.3, 30.3, 28.9, 28.0, 27.4, 27.1, 25.4, 25.3, 25.2, 25.1, 24.9, 24.0, 21.5, 21.1, 21.0, 19.4, 12.7; ^{31}P-NMR (121 MHz, $CDCl_3$) δ=148.1, 147.4; HRMS-FAB (m/z): [M+1]$^+$ calcd for $C_{54}H_{78}O_5N_2P_1$, 865.5648; found, 865.5641.

Example 5: Preparation of phosphoramidite compound of formula (IVa) used in the synthesis of bDNA

(1) DMTr protection reaction of pentaerithritol (compound 10, 11, and 12)

Pentaerithritol (1.1 g, 7.34 mmol) and DMAP (4-dimethylaminopyridine, 276 mg, 2.26 mmol) were dissolved in 15 ml of Py/DMF (2/1), DMTr-Cl (4.1 g, 12.1 mmol) was added thereto and stirred for 10 hours at room temperature. 80 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 50 ml of CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The

resulting orange colored oil residue was purified by flash chromatography (eluent – ethyl acetate : hexane = 1 : 2 (compound 10); ethyl acetate : hexane = 1 : 1 (compound 11); and CH_2Cl_2: MeOH = 10 : 1 (compound 12)) to obtain the title compound (compound 10: 2.93 g, 2.81 mmol, 70%; compound 11: 520 mg, 0.702 mmol, 11.6%; and compound 12: 395 mg, 0.901 mmol, 7.4%).

Compound 10: m.p. 96.3-97.8°C MS (FAB): m/z: 1065.3 ($M+Na^+$); IR (neat): v=3410.1, 2929.6, 1607.4, 1508.1, 1461.7, 1300.2, 1250.6, 1176.4, 1034.3 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$): δ=7.26-7.24 (m, 6H), 7.19-7.15 (m, 21H), 6.72 (d, 12H, *J*=8.9Hz), 3.76 (s, 18H), 3.59 (s, 2H), 3.32 (s, 2H); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ=158.8, 145.3, 136.3, 130.6, 128.6, 128.1, 126.9, 113.4, 86.4, 64.3, 55.5, 45.8.

Compound 11: m.p. 88.8-89.7°C. MS (FAB): m/z: 763.2 $(M+Na)^+$; IR (neat): v=3442, 1684, 1652, 1608, 1507, 1457, 1250, 1217, 1176, 1034cm^{-1}, ^{1}H-NMR (300MHz, $CDCl_3$): δ=7.38-7.36 (m, 4H), 7.29-7.20 (m, 14H), 6.80 (4, 8H, *J*=8.5Hz), 3.76 (s, 12H), 3.64 (s, 4H), 3.23 (s, 4H), 2.39 (s, 2H); ^{13}C-NMR (75.5MHz, $CDCl_3$): δ=158.0, 144.3, 135.3, 129.7, 127.7, 127.4, 126.3, 112.7, 85.8, 65.0, 62.7, 54.7, 45.0; HRMS-FAB (m/z): $[M+Na]^+$ calcd for $C_{52}H_{54}O_{10}Na$, 763.3247; found, 763.3247.

Compound 12: an oil at room temperature; MS (FAB): m/z: 461.1 ($M+Na^+$); IR (neat): v = 3734.1, 3404.7, 2927.3, 1733.7, 1607.2, 1540.8, 1508.1, 1458.0, 1300.8, 1250.1, 1176.1, 1033.3, 828.9, 754.7 cm^{-1}, ^{1}H-NMR (300MHz, $CDCl_3$): δ=7.40-7.39 (m, 2H), 7.31-7.24 (m, 7H), 6.84-6.81 (m, 4H), 3.77 (s, 6H), 3.71 (s, 6H), 3.16 (s, 2H), 2.35 (br, 2H), 1.63 (br, 1H); ^{13}C-NMR (75.5MHz, $CDCl_3$): δ=158.8, 135.7, 130.2, 128.2, 127.2, 113.5, 65.4, 64.1, 55.4, 45.5.

(2) Preparation of *O*-tri-DMTr-((2-cyanoethyl)-*N,N*-diisopropyl-phosphoramidite)-pentaerithritol (compound IVa)

Compound 10 (560 mg, 0.537 mmol) obtained in step 1 and DIPEA (187 μℓ, 1.074 mmol) were dissolved in 6 ml of THF. Chloro-(2-cyanoethyl)-*N,N*-diisopropyl-phosphine (297 μℓ, 1.34 mmol) was added dropwise thereto and stirred for 1 hour at room temperature. 10 ml of 5%

$NaHCO_3$ was added thereto and the resulting solution was extracted with 10 ml of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting yellow oil residue was purified by flash chromatography (eluent – ethyl acetate : hexane = 1 : 3) to obtain the title compound (236 mg, 0.190 mmol) as a colorless oil in a yield of 35%.

MS (FAB): m/z: 1265.6 [M+Na^+]; ^{1}H-NMR (300 MHz, $CDCl_3$): δ=7.27-7. 14 (m, 27H), 6.72-6.68 (m, 12H), 4.11 (q, 2H, *J*=6.7Hz), 3.75 (s, 18H), 3.39-3.23 (m, 8H), 2.23 (t, 2H, *J*=6.3Hz), 2.03 (s, 2H), 1.31-1.22 (m, 4H), 1.09 (d, 6H, *J*=6.7Hz), 0.95 (d, 6H, *J*=6.7Hz); ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ=157.7, 144.7, 135.8, 129.7, 127.8, 127.1, 126.0, 112.5, 85.1, 62.3, 57.7, 54.7, 42.5, 24.1, 24.0, 13.7; ^{31}P-NMR (121.5 MHz, $CDCl_3$): δ=148.9; HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{77}H_{83}N_2O_{11}P_1Na_1$, 1243.5852; found, 1243.5807.

Example 6: Preparation of phosphoramidite compound of formula (IVb) used in the synthesis of bDNA

(1) Preparation of *O*-Di-DMTr-*O*-Lev-pentaerithritol (compound 13)

Compound 11 (506 mg, 0.68 mmol) obtained in step 1 of Example 5, EDC (288 mg, 1.50 mmol), and DMAP (184 mg, 1.50 mmol) were dissolved in 14 ml of CH_2Cl_2. Luvulinic acid (77 μℓ, 0.75mmol) was added thereto and stirred for 3 hours at room temperature. 20 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 10 ml of CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting residue was purified by flash chromatography (eluent – ethyl acetate : hexane = 1 : 2) to obtain the title compound (319.7 mg, 0.37 mmol) as a yellow solid in a yield of 55%.

m.p. 55.4-56.1°C; MS (FAB): m/z: 861.3 (M+Na^+); IR (neat): v=3522.7, 3055.8, 3035.1, 3000.1, 2955.5, 2932.7, 2836.1, 1733.6, 1717.2, 1506.3, 1301.6, 1251.3, 1177.6, 1154.9, 1072.5, 1033.9 cm^{-1}; ^{1}H-NMR (300MHz, Acetone-d_6): δ=7.41-7.38 (m, 4H), 7.29-7.18 (m, 14H), 6.85-6.82 (m, 8H), 4.15 (s, 2H), 3.77 (s, 12H), 3.77 (s, 12H), 3.69-3.67 (m, 2H), 3.50 (m, 1H), 3.29 (s, 4H), 2.63 (t, 2H, *J*=6.7Hz), 2.35 (t, 2H, *J*=6.7Hz), 2.07 (s, 3H); ^{13}C-NMR (75.5MHz, $CDCl_3$): δ=205.7, 172.4, 159.0, 145.9, 136.4, 130.6, 128.0,

126.9, 126.3, 113.3, 86.2, 64.0, 61.9, 55.0, 45.5, 37.7, 28.0; HRMS-FAB (m/z): $[M+Na]^+$ calcd for $C_{52}H_{54}O_{10}Na$, 861.3615; found, 861.3617.

(2) Preparation of *O*-DMTr-*O*-di-Lev-*O*-((2-cyanoethyl)-*N,N*-diisopropyl-phosphoramidite)-pentaerithritol (compound IVb)

O-Di-DMTr-*O*-Lev-pentaerithritol (319.7 mg, 0.37 mmol) obtained in step 1 and DIPEA (260 $\mu\ell$, 1.48 mmol) were dissolved in 4 ml of THF. Chloro-(2-cyanoetliyl)-*N,N*-diisopropyl-phosphine (166 $\mu\ell$, 0.74 mmol) was added dropwise thereto and stirred for 1.5 hours at room temperature. 10 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 10 ml of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting residue was purified by flash chromatography (eluent – CH_2Cl_2 : hexane = 3 : 2) to obtain the title compound (302.8 mg, 0.29 mmol) as a yellow oil in a yield of 77%.

MS (FAB): m/z: 1040 ($M+H^+$); IR (neat): v=2964, 2931, 2932, 1736, 1720, 1607, 1581, 1508, 1463, 1444, 1250, 1177, 1032 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ=7.24 (d, 4H, *J*=7.2Hz), 7.17-7.09 (m, 14H), 6.67 (d, 8H, *J*=8.4Hz), 4.02 (q, 2H, *J*=10.7Hz), 3.68 (s, 12H), 3.66-3.37 (m, 6H), 3.16 (m, 4H), 2.50 (t, 2H, *J*=7.0Hz), 2.35 (t, 2H, *J*=6.3Hz), 2.28 (t, 2H, *J*=6.7z), 2.05 (s, 3H), 1.21 (t, 4H, *J*=5.7Hz), 1.05 (d, 6H, *J*=6.7Hz), 0.94 (d, 6H, *J*=6.7Hz); ^{13}C-NMR (75,5 MHz, $CDCl_3$): δ=172.5, 158.8, 145.2, 136.2, 130.5, 128.4, 127.9, 126.8, 117.9, 113.2, 86.0, 61.6, 60.6, 55.4, 43.3, 43.1, 38.0, 30.0, 28.0, 24.8, 24.7, 14.4, 158.0, 144.2, 135.3, 135.0, 129.7, 127.7, 127.4, 127.3, 126.3, 117.2, 112.6, 112.5, 85.8, 85.5, 62.6, 61.6, 61.4, 60.2, 57.9, 57.7, 54.7, 46.9, 43.5, 42.7, 42.5, 37.3, 30.47, 29.3, 27.3, 24.2, 24.1, 22.1, 20.7, 19.9, 19.8; ^{31}P-NMR (121.5 MHz, $CDCl_3$): 6=150.2; HRMS-FAB (m/z): $[M+H]^+$ calcd for $C_{61}H_{72}N_2O_{11}P_1Na_1$, 1039.4874; found, 1039.4877.

Example 7: Preparation of phosphoramidite compound of formula (IVc) used in the synthesis of bDNA

(1) Preparations of *O*-DMTr-*O*-Lev-pentaerithritol (compound 14) and *O*-DMTr-*O*-di-Lev-pentaerithritol (compound 15)

Compound 12 (484.3 mg, 1.10 mmol) obtained in step 1 of Example 5, EDC (253 mg, 1.32 mmol) and DMAP (162 mg, 1.32 mmol) were dissolved in 10 ml of CH_2Cl_2. Luvulinic acid (135 mg, 1.32 mmol) was added thereto and stirred for 3 hours at room temperature. 20 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 15 ml of CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting residue was purified by flash chromatography (eluent – ethyl acetate : hexane = 1 : 2 (compound 14); and ethyl acetate : hexane = 1 : 1 (compound 15)) to obtain the title compounds as yellow liquids (compound 14: 141 mg, 0.22 mmol; and compound 15: 285 mg, 0.53 mmol, 48 %), respectively.

Compound 14: MS (FAB): m/z 657.2 ($M+Na^+$); IR (neat): v=3390, 1792, 1772, 1699, 1684, 1653, 1558, 1540, 1521cm^{-1} ^{1}H-NMR (300 MHz, $CDCl_3$): δ=7.41-7.38 (m, 2H), 7.28-7.20 (m, 7H), 6.85-6.81 (m, 4H), 4.16-4.10 (m, 4H), 3.78 (s, 6H), 3.54 (d, 2H, *J*=6.8Hz), 3.14 (s, 2H), 2.69 (t, 4H, *J*=6.5Hz), 2.50-2.44 (m, 4H), 2.15 (s, 6H), 1.25 (*t*, 1H, *J*=7.14Hz); ^{13}C-NMR-DEPT (75.5 MHz, $CDC1_3$): 5=130.7 (CH_1), 128.7(CH_1), 128.5 (CH_1), 128.5 (CH_1), 113.8 (CH_1), 63.6 (CH_2), 62.6 (CH_2), 61.8 (CH_2), 55.9(CH_3), 38.6 (CH_2), 30.4 (CH_3), 28.5 (CH_2); HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{36}H_{42}O_{10}Na$, 657.2676; found, 657.2673.

Compound 15: MS (FAB): m/z 559.2 ($M+Na^+$); IR (neat): v=3400, 3179, 3084, 3056, 3001, 2929.7, 2835.8, 1716.9, 1606.4, 1508.5, 1445.2, 1380.3, 1301.3, 1250.3, 1228.2, 1177.6, 1033.9, 996.5, 949.8, 830.4, 807.2, 754.7cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$): δ=7.38-7.35 (m, 2H), 7.26-7.11 (m, 7H), 6.75 (d, 4H, *J*=8.5Hz), 4.35 (s, 2H), 4.22(s, 2H), 3.68 (s, 6H), 3.64 (s, 4H), 3.08 (s, 2H), 2.57 (t, 2H, *J*=6.6Hz), 2.37 (t, 2H, *J*=6.6Hz), 2.05 (s, 3H); ^{13}C-NMR (75.5MHz, $CDCl_3$): δ=206.2, 172.4, 157.9, 153.8, 144.4, 135.4, 129.6, 127.6, 127.3, 126.2, 112.6, 85.5, 63.4, 63.2, 61.7, 54.6, 44.3, 29.3, 27.4; HRMS-FAB (m/z): $[M+Na]^+$ calcd for $C_{31}H_{36}O_8Na$, 559.2308; found, 559.2308.

(2) Preparation of *O*-DMTr-*O*-di-Lev-*O*-((2-cyanoethyl)-*N,N*-diisopropyl-phosphoramidite)-pentaerithritol (compound IVc)

O-DMTr-*O*-Lev-pentaerithritol (compound 14) (141 mg, 0.222 mmol) obtained in step 1 and DIPEA (77 $\mu\ell$, 0.445 mmol) were dissolved in 6 ml of THF. Chloro-(2-cyanoethyl)-*N,N*-diisopropyl-phosphine (74 $\mu\ell$, 0.33 mmol) was added dropwise thereto and stirred for 30 minutes at room temperature. 10 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 10 ml of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting residue was purified by flash chromatography (eluent – ethyl acetate : hexane = 1 : 3) to obtain the title compound (114.2 mg, 0.1354 mmol) as a yellow oil in a yield of 61%.

MS (FAB): IR (neat): v=2966, 2933, 1738, 1717, 1607, 1508, 1463, 1362, 1301, 1250, 1202, 1178, 1154, 1076, 1032 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ=7.40-7.38 (m, 2H), 7.29-7.26 (m, 7H), 6.82 (d, 4H, *J* =8.7Hz), 4.17-4.12 (m, 4H), 3.78 (s, 6H), 3.73-3.65 (m, 2H), 3.61-3.51 (m, 2H), 3.15 (s, 2H), 2.68 (t, 4H, *J*=6.5Hz), 2.56 (t, 2H, *J*= 6.3Hz), 2.48 (t, 4H, *J*=6.5Hz), 2.16 (s, 6H), 1.25 (d, 2H, *J*=6.7Hz), 1.16 (d, 6H, *J*= 6.7Hz), 1.10 (d, 6H, *J*=6.7Hz); ^{13}C-NMR (75,5 MHz, $CDCl_3$): δ=205.8, 171.8, 158.0, 158.0, 144.2, 135.3, 135.0, 129.7, 127.7, 127.4, 127.3, 126.3, 117.2, 112.6, 112.5, 85.8, 85.5, 62.6, 61.6, 61.4, 60.2, 57.9, 57.7, 54.7, 46.9, 43.5, 42.7, 42.5, 37.3, 30.47, 29.3, 27.3, 24.2, 24.1, 22.1, 20.7, 19.9, 19.8; ^{31}P-NMR (121.5 MHz, $CDCl_3$): δ=150.7

Example 8: Preparation of phosphoramidite compound of formula (IVd) used in the synthesis of bDNA

(1) Preparation of *O*-DMTr-*O*-Lev-TBDMS-pentaerithritol (compound 16)

O-DMTr-*O*-di-Lev-pentaerithritol (compound 15) (213 mg, 0.40 mmol) obtained in step 1 of Example 7 and DMAP (164 mg, 1.33 mmol) were dissolved in 4 ml of THF. *Tert*-Butyldimethylsilyl chloride (65 mg, 0.44 mmol) was added thereto and stirred for 3 hours at room temperature. 10 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 20 ml of CH_2Cl_2. The organic layer was dried over $MgSO_4$

and evaporated under a reduced pressure. The resulting residue was purified by flash chromatography (eluent – ethyl acetate : hexane = 1 : 2) to obtain the title compound (119 mg, 0.18 mmol, 46%; Di-protected product: 72.8mg, 0.1mmol, 25%) as a yellow oil.

MS (FAB): m/z 673.3 ($M+Na^+$); IR (neat): v=3500.5, 2953.9, 2930.1, 2855.9, 1720.0, 1608.1, 1509.0, 1463.7, 1444.9, 1359.5, 1301.4, 1251.4, 1177.4, 1157.6, 1071.9, 1035.4, 911.9, 836.1 cm^{-1}; ^{1}H-NMR (300MHz, $CDCl_3$): δ=7.43-7.41 (m, 2H), 7.32-7.26 (m, 7H), 6.85-6.82 (m, 4H), 4.20 (d, 2H, *J*=4.0Hz), 3.79 (s, 6H), 3.66-3.64 (m, 4H), 3.15 (s, 2H), 2.70 (t, 2H, *J*=6.6Hz), 2.50 (t, 2H, *J*=6.5Hz), 2.17 (s, 3H), 0.84 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C-NMR (75.5MHz, $CDCl_3$): δ=206.6, 173.0, 158.9, 145.1, 136.2, 130,5, 128.5, 128.2, 127.2, 113.5, 86.6, 64.8, 64.0, 63.9, 62.6, 55.6, 45.5, 38.3, 30.1, 28.3, 26.2, 18.5, -5.3; HRMS-FAB (m/z): $[M+Na]^+$ calcd for, 673.3173; found, 673.3173.

(2) Preparation of *O*-DMTr-*O*-Lev-*O*-TBDMS-((2-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite)-pentaerithritol (compound IVd)

O-DMTr-*O*-Lev-TBDMS-pentaerithritol (compound 16) (134.8 mg, 0.207 mmol) obtained in step 1 and DIPEA (144 μℓ, 0.828 mmol) were dissolved in 4.2 ml of THF. Chloro-(2-cyanoethyl)-*N*,*N*-diisopropyl-phosphine (114 μℓ, 0.518 mmol) was added dropwise thereto and stirred for 1.5 hours at room temperature. 10 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 10 ml of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting residue was purified by flash chromatography (eluent – ethyl acetate : hexane = 1 : 3) to obtain the title compound (86.2 mg, 0.101 mmol) as a yellow oil in a yield of 50%.

MS (FAB): m/z: 873.33 ($M+Na^+$); IR(neat): v=2961.8, 2930.2, 2881.8, 2856.3, 1738.0, 1721.9, 1607.7, 1508.9, 1463.6, 1445.3, 1362.9, 1279.9, 1251.5, 1178.0 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ=7.41-7.38 (m, 2H), 7.29-7.17 (m, 7H), 6.79 (d, 4H, *J*=8.9Hz), 4.11-4.09 (m, 2H), 3.76 (s, 6H), 3.70-3.52 (m, 8H), 3.12 (s, 2H), 2.66-2.64 (m, 2H), 2.53-2.45 (m, 4H), 2.15 (s, 3H), 1.14 (d, 6H, *J*= 6.8Hz), 1.08 (dd, 6H, J_1=6.7Hz, J_2=1.4Hz), 0.08 (d, 9H, *J*= 1.1Hz), -0.03 (d, 6H, *J*=2.4Hz); ^{13}C-NMR(75.5 MHz, $CDCl_3$): δ=205.9,

171.9, 157.9, 144.6, 135.7, 129.7, 127.2, 126.1, 117.2, 112.5, 85.3, 63.2, 61.0, 60.4, 57.7, 54,7, 45.0, 42.6, 42.5, 37.4, 29.4, 27.3, 25.3, 24.2, 24.1, 19.9, 19.8, 17.7, -6.12; ^{31}P-NMR (121.5 MHz, $CDCl_3$): δ=150.3, 150.1; HRMS-FAB (m/z): $[M+Na]^+$ calcd for $C_{46}H_{67}N_2O_9P_1Si_1Na_1$, 873.4251; found, 873.4252.

Example 9: Preparation of phosphoramidite compound of formula (V) using dendrimer

Dendrimer compound 17 (84 mg, 0.11 mmol, Hawker, C. J.; Frkchet, J. M. J. *J. Am. Chem. SOC.112,* 7638-7647(1990)) and N-methyl morpholine (260 μℓ, 2.36 mmol) were dissolved in 4 ml of CH_3CN. Chloro-(2-cyanoethyl)-*N,N*-diisopropyl-phosphine (140 μℓ, 0.62 mmol) was added dropwise thereto and stirred for 5 minutes at room temperature. 10 ml of 5% $NaHCO_3$ was added thereto and the resulting solution was extracted with 15 ml of ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated under a reduced pressure. The resulting residue was purified by flash chromatography (eluent – ethyl acetate : hexane = 1 : 3) to obtain the title compound (96 mg, 0.10 mmol) as a pale yellow oil in a yield of 92%.

^{1}H-NMR (300 MHz, $CDCl_3$): δ=7.32-7.23 (m, 20H), 6.61 (s, 4H), 6.50 (br, 4H), 6.47 (s, 1H), 4.94 (s, 8H), 4.88 (s, 4H), 4.49 (s, 2H), 4.11 (q, 2H), 3.92 (t, 2H), 2.28 (t, 2H), 1.04(d, 6H), 0.93 (d, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ=160.9, 160.8, 140.0, 137.5, 129.3, 128.7, 128.3, 107.2, 106.5, 102.4, 70.9, 70.7, 53.0, 44.9, 44.8, 43.0, 22.5, 22.4; ^{31}P-NMR (127 MHz, $CDCl_3$): δ=150.9.

While the invention has been described with respect to the above specific embodiments, it should be recognized that various modifications and changes may be made to the invention by those skilled in the art which also fall within the scope of the invention as defined by the appended claims.